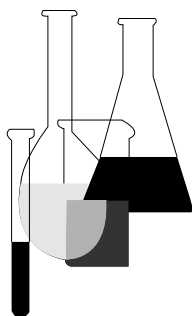




Health Effects Test Guidelines OPPTS 870.5300 *In Vitro* Mammalian Cell Gene Mutation Test



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 870.5300 In vitro mammalian cell gene mutation test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5300 Detection of gene mutations in somatic cells in culture and OECD 476, *In Vitro* Mammalian Cell Gene Mutation Test.

(b) **Introduction.** The *in vitro* mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances. Suitable cell lines include L5178Y mouse lymphoma cells, the CHO, AS52 and V79 lines of Chinese hamster cells, and TK6 human lymphoblastoid cells (see reference in paragraph (g)(1) of this guideline). In these cell lines the most commonly-used genetic endpoints measure mutation at thymidine kinase (TK) and hypoxanthine-guanine phosphoribosyl transferase (HPRT), and a transgene of xanthine-guanine phosphoribosyl transferase (XPRT). The TK, HPRT and XPRT mutation tests detect different spectra of genetic events. The autosomal location of TK and XPRT may allow the detection of genetic events (e.g., large deletions) not detected at the HPRT locus on X-chromosomes (see references in paragraphs (g)(2), (g)(3), (g)(4), (g)(5), and (g)(6) of this guideline).

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Base pair substitution mutagens are substances which cause substitution of one or several base pairs in the DNA.

Forward mutation is a gene mutation from the parental type to the mutant form which gives rise to an alteration or a loss of the enzymatic activity or the function of the encoded protein.

Frameshift mutagens are substances which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

Mutant frequency is the number of mutant cells observed divided by the number of viable cells.

Phenotypic expression time is a period during which unaltered gene products are depleted from newly mutated cells.

Relative suspension growth is an increase in cell number over the expression period relative to the negative control.

Relative total growth is an increase in cell number over time compared to a control population of cells; calculated as the product of suspen-

sion growth relative to the negative control times cloning efficiency relative to negative control.

Survival is the cloning efficiency of the treated cells when plated at the end of the treatment period; survival is usually expressed in relation to the survival of the control cell population.

Viability is the cloning efficiency of the treated cells at the time of plating in selective conditions after the expression period.

(d) **Initial considerations.** (1) In the *in vitro* mammalian cell gene mutation test, cultures of established cell lines or cell strains can be used. The cells used are selected on the basis of growth ability in culture and stability of the spontaneous mutation frequency. Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. This metabolic activation system cannot mimic entirely the mammalian *in vivo* conditions. Care should be taken to avoid conditions which would lead to results not reflecting intrinsic mutagenicity. Positive results which do not reflect intrinsic mutagenicity may arise from changes in pH, osmolality or high levels of cytotoxicity (see reference in paragraph (g)(7) of this guideline).

(2) This test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is dependent on chemical class and there is increasing evidence that there are carcinogens that are not detected by this test because they appear to act through other, non-genotoxic mechanisms or mechanisms absent in bacterial cells (see reference in paragraph (g)(6) of this guideline).

(e) **Test method—(1) Principle.** (i) Cells deficient in thymidine kinase (TK) due to the mutation $TK^{+/-} \rightarrow TK^{-/-}$ are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). Thymidine kinase proficient cells are sensitive to TFT, which causes the inhibition of cellular metabolism and halts further cell division. Thus mutant cells are able to proliferate in the presence of TFT, whereas normal cells, which contain thymidine kinase, are not. Similarly, cells deficient in HPRT or XPRT are selected by resistance to 6-thioguanine (TG) or 8-azaguanine (AG). The properties of the test substance should be considered carefully if a base analogue or a compound related to the selective agent is tested in any of the mammalian cell gene mutation tests. For example, any suspected selective toxicity by the test substance for mutant and non-mutant cells should be investigated. Thus, performance of the selection system/agent must be confirmed when testing chemicals structurally related to the selective agent (see reference in paragraph (g)(8) of this guideline).

(ii) Cells in suspension or monolayer culture are exposed to the test substance, both with and without metabolic activation, for a suitable period

of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection (see references in paragraphs (g)(9), (g)(10), (g)(11), (g)(12), and (g)(13) of this guideline). Cytotoxicity is usually determined by measuring the relative cloning efficiency (survival) or relative total growth of the cultures after the treatment period. The treated cultures are maintained in growth medium for a sufficient period of time, characteristic of each selected locus and cell type, to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted. The mutant frequency is derived from the number of mutant colonies in selective medium and the number of colonies in non-selective medium.

(2) **Description—(i) Preparations—(A) Cells.** (1) A variety of cell types are available for use in this test including subclones of L5178Y, CHO, CHO-AS52, V79, or TK6 cells. Cell types used in this test should have a demonstrated sensitivity to chemical mutagens, a high cloning efficiency and a stable spontaneous mutant frequency. Cells should be checked for *Mycoplasma* contamination and should not be used if contaminated.

(2) The test should be designed to have a predetermined sensitivity and power. The number of cells, cultures and concentrations of test substance used should reflect these defined parameters (see reference in paragraph (g)(14) of this guideline). The minimal number of viable cells surviving treatment and used at each stage in the test should be based on the spontaneous mutation frequency. A general guide is to use a cell number which is at least ten times the inverse of the spontaneous mutation frequency. However, it is recommended to utilise at least 10^6 cells. Adequate historical data on the cell system used should be available to indicate consistent performance of the test.

(B) **Media and culture conditions.** Appropriate culture media and incubation conditions (culture vessels, temperature, CO₂ concentration and humidity) should be used. Media should be chosen according to the selective systems and cell type used in the test. It is particularly important that culture conditions should be chosen that ensure optimal growth of cells during the expression period and colony forming ability of both mutant and non-mutant cells.

(C) **Preparation of cultures.** Cells are propagated from stock cultures, seeded in culture medium and incubated at 37 °C. Prior to use in this test, cultures may need to be cleansed of pre-existing mutant cells.

(D) **Metabolic activation.** Cells should be exposed to the test substance both in the presence and absence of an appropriate metabolic activa-

tion system. The most commonly used system is a co-factor-supplemented post-mitochondrial fraction (S9) prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254 (see references in paragraphs (g)(15), (g)(16), (g)(17), and (g)(18) of this guideline) or a combination of phenobarbitone and β -naphthoflavone (see references in paragraphs (g)(19) and (g)(20) of this guideline). The post-mitochondrial fraction is usually used at concentrations in the range from 1–10 percent v/v in the final test medium. The choice and condition of a metabolic activation system may depend upon the class of chemical being tested. In some cases it may be appropriate to utilize more than one concentration of post-mitochondrial fraction. A number of developments, including the construction of genetically engineered cell lines expressing specific activating enzymes, may provide the potential for endogenous activation. The choice of the cell lines used should be scientifically justified (e.g., by the relevance of the cytochrome P450 isoenzyme to the metabolism of the test substance).

(E) **Test substance/preparations.** Solid test substances should be dissolved or suspended in appropriate solvents or vehicles and diluted if appropriate prior to treatment of the cells. Liquid test substances may be added directly to the test systems and/or diluted prior to treatment. Fresh preparations should be employed unless stability data demonstrate the acceptability of storage.

(ii) **Test conditions—(A) Solvent/vehicle.** The solvent/vehicle should not be suspected of chemical reaction with the test substance and should be compatible with the survival of the cells and the S9 activity. If other than well-known solvent/vehicles are used, their inclusion should be supported by data indicating their compatibility. It is recommended that whenever possible, the use of an aqueous solvent/vehicle be considered first. When testing water-unstable substances, the organic solvents used should be free of water. Water can be removed by adding a molecular sieve.

(B) **Exposure concentrations.** (1) Among the criteria to be considered when determining the highest concentration are cytotoxicity and solubility in the test system and changes in pH or osmolality.

(2) Cytotoxicity should be determined with and without metabolic activation in the main experiment using an appropriate indicator of cell integrity and growth, such as relative cloning efficiency (survival) or relative total growth. It may be useful to determine cytotoxicity and solubility in a preliminary experiment.

(3) At least four analysable concentrations should be used. Where there is cytotoxicity, these concentrations should cover a range from the maximum to little or no toxicity; this will usually mean that the concentration levels should be separated by no more than a factor between 2 and $\sqrt{10}$. If the maximum concentration is based on cytotoxicity then it should

result in approximately 10–20 percent but not less than 10 percent relative survival (relative cloning efficiency) or relative total growth. For relatively non-cytotoxic compounds the maximum concentration should be 5 mg/ml, 5 µl/ml, or 0.01 M, whichever is the lowest.

(4) Relatively insoluble substances should be tested up to or beyond their limit of solubility under culture conditions. Evidence of insolubility should be determined in the final treatment medium to which cells are exposed. It may be useful to assess solubility at the beginning and end of the treatment, as solubility can change during the course of exposure in the test system due to presence of cells, S9, serum etc. Insolubility can be detected by using the unaided eye. The precipitate should not interfere with the scoring.

(C) **Controls.** (1) Concurrent positive and negative (solvent or vehicle) controls both with and without metabolic activation should be included in each experiment. When metabolic activation is used the positive control chemical should be one that requires activation to give a mutagenic response.

(2) Examples of positive control substances include:

Metabolic Activation condition	Locus	Chemical	CAS number
Absence of exogenous metabolic activation	HPRT	Ethylmethanesulfonate	[62–50–0]
	TK (small and large colonies).	EthylNitrosourea	[759–73–9]
		Methylmethanesulfonate	[66–27–3]
	XPRT	Ethylmethanesulfonate	[62–50–0]
Presence of exogenous metabolic activation.	HPRT	EthylNitrosourea	[759–73–9]
		3-Methylcholanthrene	[56–49–5]
		N-Nitrosodimethylamine	[62–75–9]
	TK (small and large colonies).	7,12-Dimethylbenzanthracene	[57–97–6]
		Cyclophosphamide (monohydrate).	[50–18–0]
		Benzo(a)pyrene	[(6055–19–2)]
		3-Methylcholanthrene	[50–32–8]
	XPRT	N-Nitrosodimethylamine	[56–49–5]
		(for high levels of S-9).	[62–75–9]
		Benzo(a)pyrene	[50–32–8]

(3) Other appropriate positive control reference substances may be used, e.g., if a laboratory has a historical data base on 5-Bromo 2'-deoxyuridine [CAS no. 59–14–3], this reference substance could be used as well. The use of chemical class-related positive control chemicals may be considered, when available.

(4) Negative controls, consisting of solvent or vehicle alone in the treatment medium, and treated in the same way as the treatment groups should be included. In addition, untreated controls should also be used unless there are historical control data demonstrating that no deleterious or mutagenic effects are induced by the chosen solvent.

(3) **Procedure**—(i) **Treatment with test substance.** (A) Proliferating cells should be exposed to the test substance both with and without metabolic activation. Exposure should be for a suitable period of time (usually 3 to 6 hours is effective). Exposure time may be extended over one or more cell cycles.

(B) Either duplicate or single treated cultures may be used at each concentration tested. When single cultures are used, the number of concentrations should be increased to ensure an adequate number of cultures for analysis (e.g., at least eight analysable concentrations). Duplicate negative (solvent) control cultures should be used.

(C) Gaseous or volatile substances should be tested by appropriate methods, such as in sealed culture vessels (see references in paragraphs (g)(21) and (g)(22) of this guideline).

(ii) **Measurement of survival, viability, and mutant frequency.** (A) At the end of the exposure period, cells should be washed and cultured to determine survival and to allow for expression of the mutant phenotype. Measurement of cytotoxicity by determining the relative cloning efficiency (survival) or relative total growth of the cultures is usually initiated after the treatment period.

(B) Each locus has a defined minimum time requirement to allow near optimal phenotypic expression of newly induced mutants (HPRT and XPRT require at least 6–8 days, and TK at least 2 days). Cells are grown in medium with and without selective agent(s) for determination of numbers of mutants and cloning efficiency, respectively. The measurement of viability (used to calculate mutant frequency) is initiated at the end of the expression time by plating in non-selective medium.

(C) If the test substance is positive in the L5178Y TK⁺-test, colony sizing should be performed on at least one of the test cultures (the highest positive concentration) and on the negative and positive controls. If the test substance is negative in the L5178Y TK⁺-test, colony sizing should be performed on the negative and positive controls. In studies using TK6TK⁺-, colony sizing may also be performed.

(f) **Data and reporting**—(1) **Treatment of results.** (i) Data should include cytotoxicity and viability determination, colony counts and mutant frequencies for the treated and control cultures. In the case of a positive response in the L5178Y TK⁺-test, colonies are scored using the criteria of small and large colonies on at least one concentration of the test substance (highest positive concentration) and on the negative and positive control. The molecular and cytogenetic nature of both large and small colony mutants has been explored in detail (see references in paragraphs (g)(23) and (g)(24) of this guideline). In the TK⁺-test, colonies are scored using the criteria of normal growth (large) and slow growth (small) colonies (see reference in paragraph (g)(25) of this guideline). Mutant cells

that have suffered the most extensive genetic damage have prolonged doubling times and thus form small colonies. This damage typically ranges in scale from the losses of the entire gene to karyotypically visible chromosome aberrations. The induction of small colony mutants has been associated with chemicals that induce gross chromosome aberrations (see reference in paragraph (g)(26) of this guideline). Less seriously affected mutant cells grow at rates similar to the parental cells and form large colonies.

(ii) Survival (relative cloning efficiencies) or relative total growth should be given. Mutant frequency should be expressed as number of mutant cells per number of surviving cells.

(iii) Individual culture data should be provided. Additionally, all data should be summarized in tabular form.

(iv) There is no requirement for verification of a clear positive response. Equivocal results should be clarified by further testing preferably using a modification of experimental conditions. Negative results need to be confirmed on a case-by-case basis. In those cases where confirmation of negative results is not considered necessary, justification should be provided. Modification of study parameters to extend the range of conditions assessed should be considered in follow-up experiments for either equivocal or negative results. Study parameters that might be modified include the concentration spacing, and the metabolic activation conditions.

(2) Evaluation and interpretation of results. (i) There are several criteria for determining a positive result, such as a concentration-related, or a reproducible increase in mutant frequency. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. Statistical significance should not be the only determining factor for a positive response.

(ii) A test substance, for which the results do not meet the above criteria is considered non-mutagenic in this system.

(iii) Although most studies will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgement about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated.

(iv) Positive results for an *in vitro* mammalian cell gene mutation test indicate that the test substance induces gene mutations in the cultured mammalian cells used. A positive concentration-response that is reproducible is most meaningful. Negative results indicate that, under the test conditions, the test substance does not induce gene mutations in the cultured mammalian cells used.

(3) Test report. The test report should include the following information:

- (i) Test substance:
 - (A) Identification data and CAS no., if known.
 - (B) Physical nature and purity.
 - (C) Physicochemical properties relevant to the conduct of the study.
 - (D) Stability of the test substance.
- (ii) Solvent/vehicle:
 - (A) Justification for choice of vehicle/solvent.
 - (B) Solubility and stability of the test substance in solvent/vehicle, if known.
- (iii) Cells:
 - (A) Type and source of cells.
 - (B) Number of cell cultures.
 - (C) Number of cell passages, if applicable.
 - (D) Methods for maintenance of cell cultures, if applicable.
 - (E) Absence of *Mycoplasma*.
- (iv) Test conditions:
 - (A) Rationale for selection of concentrations and number of cell cultures including e.g., cytotoxicity data and solubility limitations, if available.
 - (B) Composition of media, CO₂ concentration.
 - (C) Concentration of test substance.
 - (D) Volume of vehicle and test substance added.
 - (E) Incubation temperature.
 - (F) Incubation time.
 - (G) Duration of treatment.
 - (H) Cell density during treatment.
 - (I) Type and composition of metabolic activation system including acceptability criteria.
 - (J) Positive and negative controls.

(K) Length of expression period (including number of cells seeded, and subcultures and feeding schedules, if appropriate).

(L) Selective agent(s).

(M) Criteria for considering tests as positive, negative or equivocal.

(N) Methods used to enumerate numbers of viable and mutant cells.

(O) Definition of colonies of which size and type are considered (including criteria for “small” and “large” colonies, as appropriate).

(v) Results:

(A) Signs of toxicity.

(B) Signs of precipitation.

(C) Data on pH and osmolality during the exposure to the test substance, if determined.

(D) Colony size if scored for at least negative and positive controls.

(E) Laboratory’s adequacy to detect small colony mutants with the L5178Y TK[±] system, where appropriate.

(F) Dose-response relationship, where possible.

(G) Statistical analyses, if any.

(H) Concurrent negative (solvent/vehicle) and positive control data.

(I) Historical negative (solvent/vehicle) and positive control data with ranges, means, and standard deviations.

(J) Mutant frequency.

(vi) Discussion of the results.

(vii) Conclusion.

(g) **References.** The following references should be consulted for additional background information on this test guideline.

(1) Moore, M.M., DeMarini, D.M., DeSerres, F.J., and Tindall, K.R. (Eds.) Banbury Report 28: Mammalian Cell Mutagenesis, Cold Spring Harbor Laboratory (New York, New York, 1987).

(2) Chu, E.H.Y. and Malling, H.V. Mammalian Cell Genetics. II. Chemical Induction of Specific Locus Mutations in Chinese Hamster Cells *In Vitro*, *Proceedings of the National Academy of Science USA*, 61, 1306–1312 (1968).

- (3) Liber, H.L. and Thilly, W.G. Mutation Assay at the Thymidine Kinase Locus in Diploid Human Lymphoblasts. *Mutation Research* 94, 467–485 (1982).
- (4) Moore, M.M. et al. Differential Mutant Quantitation at the Mouse Lymphoma TK and CHO HGPRT Loci. *Mutagenesis* 4, 394–403 (1989).
- (5) Aaron, C.S. and Stankowski, Jr., L.F. Comparison of the AS52/XPRT and the CHO/HPRT Assays: Evaluation of Six Drug Candidates. *Mutation Research* 223, 121–128 (1989).
- (6) Aaron, C.S. et al. Mammalian Cell Gene Mutation Assays Working Group Report. Report of the International Workshop on Standardization of Genotoxicity Test Procedures. *Mutation Research* 312, 235–239 (1994).
- (7) Scott, D. et al. Genotoxicity Under Extreme Culture Conditions. A report from ICPEMC Task Group 9. *Mutation Research* 257, 147–204 (1991).
- (8) Clive, D. et al. Specific Gene Mutations in L5178Y Cells in Culture. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research* 115, 225–251 (1983).
- (9) Li, A.P. et al. A Review and Analysis of the Chinese Hamster Ovary/Hypoxanthine Guanine Phosphoribosyl Transferase System to Determine the Mutagenicity of Chemical Agents: A Report of Phase III of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research* 196, 17–36 (1988).
- (10) Li, A.P. et al. A Guide for the Performance of the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine Phosphoribosyl Transferase Gene Mutation Assay. *Mutation Research* 189, 135–141 (1987).
- (11) Liber, H.L., Yandell, D.W., and Little, J.B. A Comparison of Mutation Induction at the tk and hprt Loci in Human Lymphoblastoid Cells; Quantitative Differences are Due to an Additional Class of Mutations at the Autosomal TK Locus. *Mutation Research* 216, 9–17 (1989).
- (12) Stankowski, L.F. Jr., Tindall, K.R., and Hsie, A.W. Quantitative and Molecular Analyses of Ethyl Methanesulfonate- and ICR 191-Induced Molecular Analyses of Ethyl Methanesulfonate- and ICR 191-Induced Mutation in AS52 Cells. *Mutation Research* 160, 133–147 (1986).
- (13) Turner, N.T., Batson, A.G., and Clive, D. Procedures for the L5178/TK^{+/−} TK^{+/−}-Mouse Lymphoma Cell Mutagenicity Assay. (Eds.) Kilbey, B.J. et al. Handbook of Mutagenicity Test Procedures (Elsevier Science Publishers, New York, 1984) pp. 239–268.

- (14) Arlett, C.F. et al. Mammalian Cell Gene Mutation Assays Based Upon Colony Formation. (Ed.) Kirkland, D.J. Statistical Evaluation of Mutagenicity Test Data (Cambridge University Press, 1989) pp. 66–101.
- (15) Abbondandolo, A. et al. Induction of 6-Thioguanine-Resistant Mutants in V79 Chinese Hamster Cells by Mouse-Liver Microsome-Activated Dimethylnitrosamine. *Mutation Research* 46, 365–373 (1977).
- (16) Ames, B.N., McCann, J., and Yamasaki, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. *Mutation Research* 31, 347–364 (1975).
- (17) Clive, D. et al. Validation and Characterization of the L5178Y/TK⁺/Mouse Lymphoma Mutagen Assay System. *Mutation Research* 59, 61–108 (1979).
- (18) Maron, D.M. and Ames, B.N. Revised Methods for the Salmonella Mutagenicity Test. *Mutation Research* 113, 173, 215 (1983).
- (19) Elliott, B.M. et al. Alternatives to Aroclor 1254-Induced S9 in *In Vitro* Genotoxicity Assays. *Mutagenesis* 7, 175–177 (1992).
- (20) Matsushima, T. et al. A Safe Substitute for Polychlorinated Biphenyls as an Inducer of Metabolic Activation Systems. (Eds.) de Serres, F.J., Fouts, J.R., Bend, J.R., and Philpot, R.M. *In Vitro Metabolic Activation in Mutagenesis Testing* (Elsevier, North-Holland, 1976) pp. 85–88.
- (21) Krahn, D.F., Barsky, F.C., and McCooey, K.T. CHO/HGPRT Mutation Assay: Evaluation of Gases and Volatile Liquids. (Eds.) Tice, R.R., Costa, D.L., and Schaich, K.M. *Genotoxic Effects of Airborne Agents*. (New York, Plenum, 1982) pp. 91–103.
- (22) Zamora, P.O. et al. Evaluation of an Exposure System Using Cells Grown on Collagen Gels for Detecting Highly Volatile Mutagens in the CHO/HGPRT Mutation Assay. *Environmental Mutagenesis* 5, 795–801 (1983).
- (23) Applegate, M.L. et al. Molecular Dissection of Mutations at the Heterozygous Thymidine Kinase Locus in Mouse Lymphoma Cells. *Proceedings of the National Academy of Science USA*, 87, 51–55 (1990).
- (24) Moore, M.M. et al. Analysis of Trifluorothymidine-Resistant (TFT^r) Mutants of L5178Y/TK⁺/Mouse Lymphoma Cells. *Mutation Research* 151, 161–174 (1985).
- (25) Yandell, D.W., Dryja, T.P., and Little J.B. Molecular Genetic Analysis of Recessive Mutations at a Heterozygous Autosomal Locus in Human Cells. *Mutation Research* 229, 89–102 (1990).
- (26) Moore, M.M. and Doerr, C.L. Comparison of Chromosome Aberration Frequency and Small-Colony TK-Deficient Mutant Frequency in

L5178Y/TK⁺-3.7.2C Mouse Lymphoma Cells. *Mutagenesis* 5, 609–614 (1990).